Generation of integration-free induced pluripotent stem cells from healthy individuals

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DOI: https://doi.org/10.1016/j.scr.2021.102269

Stem Cell Research 53 (2021) 102269

Contents lists available at ScienceDirect

Stem Cell Research

journal homepage: www.elsevier.com/locate/scr

Lab Resource: Multiple Cell Lines

Generation of integration-free induced pluripotent stem cells from healthy individuals

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A B S T R A C T

Ten human induced pluripotent stem cell (iPSC) lines have been derived from five healthy controls matched to a study including Attention-Deficit Hyperactivity Disorder patients (ADHD). Both female and male children and adolescents aged 6–18 years were recruited. Isolated keratinocyte cells from the participants were reprogrammed into iPSCs using non-integrating Sendai virus to deliver the reprogramming factors Oct3/4, Sox2, Klf4 and cMyc.

1. Resource table:

Unique stem cell lines identifier	*TMPi001-A* *TMPi001-B* *TMPi002-A* *TMPi002-B* *TMPi003-A* *TMPi003-B* *TMPi004-A* *TMPi004-B* *TMPi005-A* *TMPi005-B*
Alternative names of stem cell lines	*KO-001 c6 (TMPi001-A)* *KO-001 c9 (TMPi001-B)* *KO-003 c14 (TMPi002-A)* *KO-003 c20 (TMPi002-B)* *KO-005 c12 (TMPi003-A)* *KO-005 c13 (TMPi003-B)* *KO-008 c13 (TMPi004-A)* *KO-008 c44 (TMPi004-B)* *KO-011 c6 (TMPi005-A)* *KO-011 c10 (TMPi005-B)*
Institution	Psychiatric University Hospital Zurich, Department of Child and Adolescent Psychiatry and Psychotherapy, University of Zurich.
Contact information of distributor	Prof. Dr. Edna Grünblatt (edna.gruenblatt@kjpd.uzh.ch)
Type of cell lines	iPSC
Origin	Human
Cell Source	Keratinocytes
Clonality	Clonal
Method of reprogramming	Sendai virus transduction
Multiline rationale	Control and disease pair (Grossmann et al., 2021)
Gene modification	NO
Type of modification	N/A
Associated disease	Healthy controls
Gene/locus	N/A
Method of modification	N/A
Name of transgene or resistance	N/A
Inducible/constitutive system	N/A
Date archived/stock date	March 2020
Cell line repository/bank	N/A
Ethical approval	Cantonal Ethics Committee (BASEC-Nr.-2016-00101 & BASEC-Nr.-201700825)

2. Resource utility

Induced pluripotent stem cells (iPSCs) are a powerful tool in disease modelling by maintaining the genetic background from somatic cells of

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https://doi.org/10.1016/j.scr.2021.102269
Received 8 February 2021; Received in revised form 8 February 2021; Accepted 18 February 2021
Available online 24 February 2021

Table 1
Summary of lines.

iPSC line names	Abbreviation in figures	Gender	Age	Ethnicity	Genotype of locus	Disease
TMPi001-A	*KO-001 c6*	Male	15	Caucasian	N/A	Healthy controls
TMPi001-B	*KO-001 c9*	Male	15	Caucasian	N/A	Healthy controls
TMPi002-A	*KO-003 c14*	Female	10	Caucasian	N/A	Healthy controls
TMPi002-B	*KO-003 c20*	Female	10	Caucasian	N/A	Healthy controls
TMPi003-A	*KO-005 c12*	Male	16	Caucasian	N/A	Healthy controls
TMPi003-B	*KO-005 c13*	Male	16	Caucasian	N/A	Healthy controls
TMPi004-A	*KO-008 c13*	Female	8	Caucasian	N/A	Healthy controls
TMPi004-B	*KO-008 c44*	Female	8	Caucasian	N/A	Healthy controls
TMPi005-A	*KO-011 c6*	Male	16	Caucasian	N/A	Healthy controls
TMPi005-B	*KO-011 c10*	Male	16	Caucasian	N/A	Healthy controls

individuals. Molecular and cellular analysis using iPSC-derived neural cells allows to elucidate differences between patients (e.g. ADHD) and healthy controls after iPSC generation and characterization to guarantee viable and trustworthy research.

3. Resource details

ADHD is one of the most complex and heterogeneous neurodevelopmental disorders, with a worldwide prevalence of over 5% and a high heritability rate of 80% (Polanczyk et al., 2007). Disease modelling through iPSCs derived from ADHD patients is crucial to understand the underlying molecular mechanisms involved in ADHD and establishing matching control lines is equally important for such research development. In this paper, iPSC lines from five healthy controls were generated using the non-integrative Sendai virus (SeV) transduction. These control cell lines match the ADHD cell lines reported by Grossmann and colleagues (Grossmann et al., 2021).

Keratinocytes from plucked hair were used as primary source for reprogramming (Table 1). The characterization of the derived iPSC lines is summarized in Table 2. By phase contrast microscopy, iPSC lines showed formation of well-delimited colonies, formed by small round cells with high nucleus:cytoplasm ratio (Fig. 1A, LM: light microscopy, scale bar: 200 μm). To biologically characterize the lines as pluripotent cells, gene and protein expression of pluripotency markers was also assessed through quantitative real-time PCR (qRT-PCR) and immunocytochemistry assays, respectively. All cell lines exhibited OCT4, SOX2, SSEA4 and TRA-1-60 expression in the latter (Fig. 1A, LM: light microscopy, scale bar: 200 μm) and positive expression of LIN28A, NANOG, OCT4 and SOX2 in the former (Fig. 1E). Additionally, possible mycoplasma contamination was assessed in a conventional PCR and demonstrated negative detection. The 1.2% agarose gel was cropped to separate controls from ADHD lines tested at the same time (Fig. 1B). All iPSCs were analysed regarding their potential to become embryoid bodies (EBs) (Fig. 1A, LM: light microscopy, scale bar: 200 μm) and their

Table 2
Characterization and validation.

Classification	Test	Result	Data
Morphology	Light microscopy	Normal morphology in which iPSCs show round morphology, aggregation in colonies with well-delimited borders	Fig. 1, panel A
Phenotype	Qualitative analysis by immunocytochemistry	Positive protein expression of pluripotency markers (OCT4, SOX2, SSEA4, and TRA-1-60)	Fig. 1, panel A
	Quantitative analysis	Positive expression of pluripotency genes (LIN28, NANOG, OCT4 and SOX2)	Fig. 1, panel E
Genotype	Genetic integrity analysis between saliva and iPSC DNA CNVs using genome-wide association array	KO-001 c6: 46XY KO-001 c9: 46XY KO-003 c14: 46XX KO-003 c20: 46XX KO-005 c12: 46XY KO-005 c13: 46XY KO-008 c13: 46XX KO-008 c44: 46XX KO-011 c6: 46XY KO-011 c10: 46XY	Fig. 1, panel C and supplementary
Identity	Infinium global screening array (Illumina)	N/A N/A	N/A Submitted in archive with journal
Mutation analysis (IF APPLICABLE)	Sequencing	N/A	N/A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	Negative	Fig. 1, item B
Differentiation potential	Embryoid body	Embryoid body formation from all cell lines with positive expression of AFP (endoderm), SOX2 (ectoderm) and FLK1 (mesoderm)	Fig. 1, panels A and F
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Saliva genotyping	DNA analysis	Not shown but available with author
	HLA tissue typing	N/A	N/A

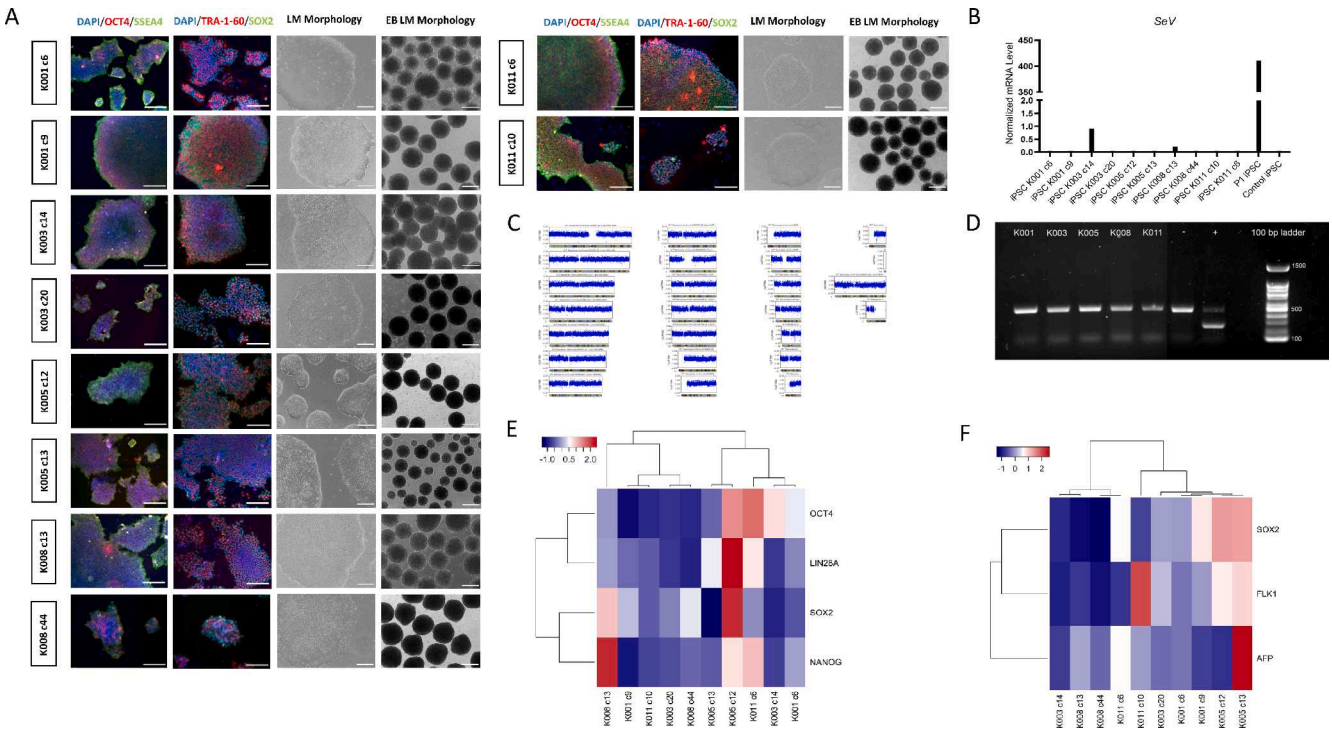

Fig. 1. Quality control of generated induced pluripotent stem cells (iPSCs) from 10 control lines. A) Light microscopy images showing morphology of all iPSCs (first column) and positive expression of pluripotent markers (OCT4, SSEA4, TRA-1-60 and SOX2) by immunocytochemistry (second and third column). Embryoid Bodies (EBs) morphology is also displayed (fourth column). B) Absence of mycoplasma in the supernatant of all iPSCs was demonstrated in an 1.2% agarose gel following DNA amplification by conventional PCR. C) Possible genetic aberrations caused by the reprogramming process were absent, as represented by the karyotype from KO-001 c6 (TMPi001-A) (for all lines see supplementary material). D) Sendai virus traces in culture were assessed by quantitative real time PCR (qRT-PCR) and compared to a positive control (P1 iPSC). E) Hierarchical gene expression analysis of pluripotency genes between iPSC lines, assessed by qRT-PCR. F) Hierarchical gene expression of endodermal (AFP), mesodermal (FLK1) and ectodermal (SOX2) markers in EBs, assessed by qRT-PCR.

potential to differentiate into the three germ layers (ectoderm, mesodermal and endoderm) by expression analysis of specific markers (Fig. 1F). Regular karyograms without significant genomic aberrations were also obtained to prove that the reprogramming process has not compromised our study (Fig. 1C shows K001 i6; see supplementary file for all lines). Possible remaining SeV was verified by qRT-PCR and demonstrated that the only cell line with minimal SeV traces (relative expression = 0.22%) was K003 c14, compared to the positive control (P1 iPSC) (relative expression = 100%) (Fig. 1D). The negative control (control iPSC) showed no expression, as expected (Fig. 1D).

4. Materials and methods

4.1. Subject recruitment

Two female and three male healthy individuals have been recruited by the Department of Child and Adolescent Psychiatry and Psychotherapy, University Hospital of Psychiatry Zurich, University of Zurich, to be part of a disease modelling study of ADHD (Grossmann et al., 2021). In contrast with ADHD patients, healthy controls did not show any psychiatric disorder, according to ICD-10 or DSM-5 (see details under supplementary material).

4.2. Reprogramming

Patient-specific keratinocytes were used as primary source for iPSCs generation by Sendai virus transduction (CytoTune™-iPS 2.0 kit by Invitrogen – Thermo Fisher Scientific), carrying polycistronic Klf4-Oct3/4-Sox2 (KOS), cMyc and Klf4. The reprogramming protocol was performed according to Re et al. (2018).

Generated iPSCs were cultivated on Vitronectin-coated wells in E8 medium and passaged every 3–4 days at a 1:3 ratio by Versene (Gibco). The iPSCs were expanded until at least passage 10 for quality control (QC).

4.3. Immunocytochemistry

Protein expression of OCT4, SSEA4, TRA-1-60 and SOX2 was assessed by the Pluripotent Stem Cell 4-Marker Immunocytochemistry Kit (Invitrogen™), according to the manufacturer's instructions. Antibodies used are listed in Table 3.

4.4. Real-time quantitative PCR analysis

Expression of pluripotency genes was determined by qRT-PCR using the QuantiFast® SYBR® Green PCR kit (Qiagen) from cDNA. RNA was extracted from iPSCs using the RNeasy® Plus Mini kit (Qiagen) and reverse transcription from 500 ng RNA into cDNA was performed by iScript™ cDNA Synthesis Kit (Bio-Rad). cDNA production and amplification of genes of interest (GOIs) and reference genes (RGs) were performed on the CFX384 thermal cycler using the primers detailed in Table 3. PCR efficiency was calculated by LinRegPCR (version 2020.0.0.3), while normalization of GOIs' mRNA levels in relation to RGs was accomplished by Biogazelle qBasePLUS2 software (version 2.3) (ACTB and HMBS for iPSCs, ACTB and GAPDH for EBs). The heatmap plot was generated by the package heatmap3 in RStudio (version 1.1.423).

Table 3
Reagents details.

Antibodies used for immunocytochemistry/flow-citometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency Markers	Rabbit anti-OCT4	1:200	Thermo Fisher Scientific Cat# A24867, RRID: AB_2650999
Pluripotency Markers	Mouse IgG3 anti-SSEA4	1:100	Thermo Fisher Scientific Cat# A24866, RRID: AB_2651001
Pluripotency Markers	Rat anti-SOX2	1:100	Thermo Fisher Scientific Cat# A24759, RRID: AB_2651000
Pluripotency Markers	Mouse IgM anti-TRA-1-60	1:100	Thermo Fisher Scientific Cat# A24868, RRID: AB_2651002
Secondary antibodies	Alexa Fluor™ 555 donkey anti-rabbit	1:250	Thermo Fisher Scientific Cat# A24869, RRID: AB_2651006
Secondary antibodies	Alexa Fluor™ 488 goat anti-mouse IgG3	1:250	Thermo Fisher Scientific Cat# A24877, RRID: AB_2651008
Secondary antibodies	Alexa Fluor™ 488 donkey anti-rat	1:250	Thermo Fisher Scientific Cat# A24876, RRID: AB_2651007
Secondary antibodies	Alexa Fluor™ 555 goat anti-mouse IgM	1:250	Thermo Fisher Scientific Cat# A24871, RRID: AB_2651009
Primers			
	Target	Forward/Reverse primer (5′-3′)	
Pluripotency Markers (qPCR)	*Sendai Virus (SeV)*	F: GGATCACTAGGTGATATCGAGC R: ACCAGACAAGAGTTTAAGAGTATGTATC	
Pluripotency Markers (qPCR)	*LIN28A*	F: AGCGCAGATCAAAAGGAGACA R: CCTCTCGAAAGTAGGTTGGCT	
Pluripotency Markers (qPCR)	*OCT4*	F: GGAGGAAGCTGACAACAATGAAA R: GGCCTGCACGAGGGTTT	
Pluripotency Markers (qPCR)	*NANOG*	F: TACCTCAGCCTCCAGCAGAT R: CTTCTGCGTCACACCATTGC	
Pluripotency and Embryoid Body Markers (qPCR)	*SOX2*	F: TGCGAGCGCTGCACAT R: TCATGAGCGTCTTGGTTTTCC	
Embryoid Body Markers (qPCR)	*AFP*	F: AAATGCGTTTCTCGTTGCTT R: GCCACAGGCCAATAGTTTGT	
Embryoid Body Markers (qPCR)	*FLK1*	F: TGATCGGAAATGACACTGGA R: CACGACTCCATGTTGGTCAC	
House-Keeping Genes (qPCR)	*HMBS*	According to manufacturer Qiagen 249,900 (QT00014462)	
House-Keeping Genes (qPCR)	*ACTB*	According to manufacturer Qiagen 249,900 (QT00095431)	
House-Keeping Genes (qPCR)	*GAPDH*	According to manufacturer Qiagen 249,900 (QT00079247)	

4.5. Detection of SeV genome

Presence of possible remaining SeV was tested in culture by using *SeV* primers in qRT-PCR (Table 3). RNA from an iPSC at passage one and a commercial human episomal iPSC line (A18945, Thermo Fisher Scientific) were used as positive and negative controls, respectively.

4.6. Mycoplasma testing

Possible mycoplasma contamination was assessed in the supernatant of iPSCs at passage eleven and above by the LookOut Mycoplasma PCR Detection Kit (Sigma-Aldrich), according to manufacturer's instructions. C1000™/CFX96™ Thermal Cycler was used for DNA amplification and amplicons were loaded on a 1.2% agarose gel containing HDGreen Plus (INTAS, Germany) and run at 100 V for 30 min. The negative internal control sample showed a band at 480 bp, while positive bands were expected at 260 bp.

4.7. Generation of embryoid bodies (EBs)

EBs were formed after iPSC culture in AggreWells with E8/PVA medium for 48 h (Lin and Chen, 2014). RNA extraction was then performed and 1 μg RNA was used for expression analysis of specific markers (Table 3).

4.8. Genotyping analysis

DNA from saliva and iPSCs were individually extracted with the GeneFix™ saliva-Prep DNA kit (Isohelix) and DNeasy® Blood & Tissue kit (Qiagen), respectively. Samples were sent for genotyping with Infinium Global Screening Array (GSA, Illumina). Analysis and comparison between the genotypes were made with the Genome Viewer function from the GenomeStudio software (version 2.0).

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

These cell lines belong to a research study supported by the PUK Forschungsfonds Nr. 8702 "Fonds für wissenschaftliche Zwecke im Interesse der Heilung von psychiatrischen Krankheiten".

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scr.2021.102269.

References

Grossmann, L., Yde Ohki, C.M., Döring, C., Hoffmann, P., Herms, P., Werling, A.M., Walitza, S., Grünblatt, E., 2021. Generation of integration-free induced pluripotent stem cell lines from four pediatric ADHD patients. Stem Cell Res. https://doi.org/10.1016/j.scr.2021.102268. In press.

Lin, Y., Chen, G., 2014. Embryoid body formation from human pluripotent stem cells in chemically defined E8 media. Harvard Stem Cell Institute, StemBook [Internet], pp. 1–4.

Polanczyk, G., et al., 2007. The Worldwide Prevalence of ADHD: A Systematic Review and Metaregression Analysis. Am. J. Psychiatry 7.

Re, S., et al., 2018. Improved generation of induced pluripotent stem cells from hair derived keratinocytes – a tool to study neurodevelopmental disorders as ADHD. Front. Cell. Neurosci. 12, 321. https://doi.org/10.3389/fncel.2018.00321.